J. Parasitol., 91(1), 2005, pp. 69–72

FIRST BIOLOGIC AND GENETIC CHARACTERIZATION OF *TOXOPLASMA GONDII* ISOLATES FROM CHICKENS FROM AFRICA (DEMOCRATIC REPUBLIC OF CONGO, MALI, BURKINA FASO, AND KENYA)

J. P. Dubey, S. Karhemere*, E. Dahl†, C. Sreekumar, A. Diabaté‡, K. R. Dabiré‡, M. C. B. Vianna, O. C. H. Kwok, and T. Lehmann†

United States Department of Agriculture, Agricultural Research Service, Animal and Natural Resources Institute, Animal Parasitic Diseases Laboratory, Building 1001, Beltsville, Maryland 20705-2350. *e-mail: jdubey@anri.barc.usda.gov*

ABSTRACT: The prevalence of *Toxoplasma gondii* in free-ranging chickens (*Gallus domesticus*) is a good indicator of the prevalence of *T. gondii* oocysts in the soil because chickens feed from the ground. In the present study, prevalence of *T. gondii* in chickens from Democratic Republic of Congo, Mali, Burkina Faso, and Kenya is reported. The prevalence of *T. gondii* antibodies in sera of 50 free-range chickens from Congo was 50% based on the modified agglutination test (MAT); antibody titers were 1:5 in 7, 1:10 in 7, 1:20 in 6, 1:40 in 1, and 1:160 or more in 4 chickens. Hearts, pectoral muscles, and brains of 11 chickens with titers of 1:20 or more were bioassayed individually in mice; *T. gondii* was isolated from 9, from the hearts of 9, brains of 3, and muscles of 3 chickens. Tissues of each of the 14 chickens with titers of 1:5 or 1:10 were pooled and bioassayed in mice; *T. gondii* was isolated from 1 chicken with a titer of 1:10. Tissues from the remaining 25 seronegative chickens were pooled and fed to 1 *T. gondii*–free cat. Feces of the cat were examined for oocysts, but none was seen. The results indicate that *T. gondii* localizes in the hearts more often than in other tissues of naturally infected chickens. Genotyping of these 10 isolates using the SAG2 locus indicated that 8 were isolates were type III, 1 was type II, and 1 was type I. Two isolates (1 type I and 1 type III) were virulent for mice. *Toxoplasma gondii* was isolated by mouse bioassay from a pool of brains and hearts of 5 of 48 chickens from Mali and 1 of 40 chickens from Burkina Faso; all 6 isolates were avirulent for mice. Genetically, 4 isolates were type III and 2 were type II. Sera were not available from chickens from Mali and Burkina Faso. *Toxoplasma gondii* antibodies (MAT 100 or more) were found in 4 of 30 chickens from Kenya, and *T. gondii* was isolated from the brain of 1 of 4 seropositive chickens; this strain was avirulent for mice and was type II. This is the first report on isolation and genotyping of *T. gondii* from any source from these 4 countries in Africa.

Toxoplasma gondii infections are widely prevalent in human beings and other animals worldwide (Dubey and Beattie, 1988). Humans become infected postnatally by ingesting tissue cysts from undercooked meat, consuming food or drink contaminated with oocysts, or by accidentally ingesting oocysts in the environment. However, only a small percentage of exposed adult humans develop clinical signs. It is not known whether the severity of toxoplasmosis in immunocompetent persons is due to the parasite strain, host variability, or to other factors.

Toxoplasma gondii isolates have been classified in 3 genetic types (I, II, and III) based on restriction fragment length polymorphism (RFLP) (Howe and Sibley, 1995; Howe et al., 1997). It has been suggested that type I strains or recombinants of types I and III are more likely to result in clinical ocular toxoplasmosis (Howe et al., 1997; Fuentes et al., 2001; Grigg et al., 2001; Aspinall et al., 2003), but genetic characterization has been limited essentially to isolates from patients ill with toxoplasmosis. Unlike these reports, Ajzenberg et al. (2002) found that most (73 of 86) isolates from cases of congenital toxoplasmosis in humans from France were type II. Nothing is known of the genetic diversity of *T. gondii* isolates circulating in the general human population. In animals, most isolates of *T. gondii* were type II or type III, irrespective of clinical status (Howe and Sibley, 1995; Mondragon et al., 1998; Owen and Trees, 1999; Jungersen et al., 2002). *Toxoplasma gondii* isolates differ markedly in their virulence to outbred mice. Type I isolates are more virulent to mice than types II and III. Because chickens become infected mostly by feeding from ground contaminated with oocysts, prevalence of *T. gondii* in chickens is a good indicator of the strains prevalent in their environment (Ruiz and Frenkel, 1980).

Recently, we found that 70% of 73 *T. gondii* isolates obtained from asymptomatic free-range chickens from Brazil were type I (Dubey et al., 2002; Dubey, Graham, da Silva et al., 2003; Dubey, Navarro et al., 2003), whereas samples from the United States and Egypt were predominantly either type II or type III but not type I (Dubey, Graham, Dahl, Hilali et al., 2003; Dubey, Graham, Dahl, Sreekumar et al., 2003). The type II isolates of *T. gondii* have not been found in chickens from Brazil. All 3 types were found in chickens from Argentina (Dubey, Venturini et al., 2003) and Mexico (Dubey, Morales et al., 2004). Nothing is known of the characteristics of isolates of *T. gondii* from animals or humans from Africa, except Egypt. In this article, we report isolation and genotyping of *T. gondii* from chickens from the Democratic Republic of Congo (DROC), Mali, Kenya, and Burkina Faso. In addition, the distribution of *T. gondii* in the heart, brain, and pectoral muscles of chickens from DROC was compared.

MATERIALS AND METHODS

Naturally infected chickens

Chickens from DROC: The chickens (n = 50) were from households near Kinshasa. Distances between the properties, where chickens were located, were recorded (Table I). Chickens were purchased and killed by cervical dislocation on 15 January 2004. Samples of serum, heart, pectoral muscle, and brain from each chicken were sent cold by air to Beltsville, Maryland. Five days elapsed between killing of chickens and receipt of samples at Beltsville, but the samples were received in excellent condition.

Chickens from Mali: Chickens (n = 48) were purchased from the local market. Therefore, no further information was available about them. They were killed on 17 January (nos. 1–11) or 24 January 2003 (nos. 12–48), and their heads and heart were sent by air to Beltsville. The samples were received at Beltsville, Maryland on 4 February 2003 and were partially autolyzed.

Received 2 June 2004; revised 8 June 2004; accepted 9 June 2004.
* B.P. 1197, Kinshasa Gombe, Democratic Republic of the Congo.
† Division of Parasitic Diseases, Centers for Disease Control and Prevention, 4770 Buford Highway, MS: F22, Chamblee, Georgia 30341.
‡ IRSS/Centre Muraz Laboratoire de Parasitologie/Entomologie, BP 390 Bobo-Dioulasso, Burkina Faso.

TABLE I. Isolation of *Toxoplasma gondii* from tissues of seropositive chickens from the Democratic Republic of Congo.

Chicken no.	Distance among chicken owners	District	Titer	Isolation in mice*						Genotype (isolate designation)
				Heart		Muscle		Brain		
				No. infected	No. died	No. infected	No. died	No. infected	No. died	
1	1 and 5, 3 km	Ngaliema	160	5	5	5	5	0	0	III (TgCkDROC-1)
5	5 and 6, 1 km	Ngaliema	160	4	0	1	0	0	0	III (TgCkDROC-2)
6	6 and 9, 3 km	Ngaliema	20	0	0	0	0	0	0	NA†
9	9 and 11, 12 km	Ngaliema	20	1	0	0	0	0	0	III (TgCkDROC-3)
11	11 and 17, 9 km	Ngaliema	20	0	0	0	0	0	0	NA†
17	17 and 19, 25 km	Kintambo	160	5	5	0	0	3	0	I (TgCkDROC-4)
19	19 and 27, 5 km	Masina	40	3	0	3	0	0	0	III (TgCkDROC-5)
27	27 and 34, 6 km	Masina	160	4	0	0	0	1	0	III (TgCkDROC-6)
34	34 and 35, 7 km	N'djili	20	3	0	0	0	0	0	III (TgCkDROC-7)
35	35 and 47, 20 km	Limete	20	5	0	0	0	5	0	III (TgCkDROC-8)
47	47 and 1, 7 km	Masina	20	5	0	0	0	0	0	III (TgCkDROC-9)

* Five mice inoculated with each tissue.
† Not applicable.

Chickens from Burkina Faso: Chickens (n = 41) were purchased from the local market, and thus no other information was available. They were killed on 30 August 2003, and the heads and hearts of 40 chickens were received at Beltsville 5 days later and were partially autolyzed.

Chickens from Kenya: Serum samples and heads from 30 chickens were obtained from a slaughterhouse in Kisumu, Kenya, on 26 June 2000 and transported to Beltsville. Twelve days elapsed between mouse bioassay and killing of chickens, and tissues were autolyzed.

Serological examination

Sera of chickens were tested for *T. gondii* antibodies using 4 serum dilutions, 1:5, 1:10, 1:20, and 1:200, with the modified agglutination test (MAT) as described by Dubey and Desmonts (1987). After the completion of the bioassays, sera from all seropositive chickens were rerun using 2-fold dilutions from 1:5 to 1:320.

Bioassay of chickens for infection

Chickens from DROC: Tissues of all chickens were bioassayed for *T. gondii* infection. Brains, pectoral muscles, and hearts of seropositive (MAT 1:20 or more) chickens were each bioassayed individually in outbred female Swiss Webster mice obtained from Taconic Farms, Germantown, New York, as described (Dubey et al., 2002). Each tissue was homogenized individually, digested in acidic pepsin, washed, and homogenate inoculated subcutaneously (s.c.) into 5 mice; in total, 15 mice were inoculated with tissues of each chicken. Tissues of chickens with titers of 1:5 or 1:10 were pooled for each chicken, digested in pepsin, and bioassayed in 5 mice. Tissues from 25 seronegative chickens were pooled and fed to 1 *T. gondii*–free cat for a period of 3 days (Dubey et al., 2002). Feces from the cat were examined for shedding of *T. gondii* oocysts 3–14 days after ingestion of chicken tissues as previously described (Dubey, 1995).

Chickens from Mali and Burkina Faso: Brains and hearts from each chicken were pooled, digested in pepsin, and the homogenates were bioassayed in mice.

Chickens from Kenya: Brains from 4 seropositive chickens were homogenized in saline, centrifuged, and the sediment was inoculated s.c. into 5 mice each.

Tissue imprints of mice that died were examined for *T. gondii* tachyzoites or tissue cysts. Survivors were bled on day 43 postinoculation (PI), and a 1:25 dilution of serum from each mouse was tested for *T. gondii* antibodies with the MAT. Mice were killed 45 days PI, and their brains were examined for tissue cysts as described (Dubey and Beattie, 1988). The inoculated mice were considered infected with *T. gondii* when tachyzoites or tissue cysts were found in tissues. A sample of the mouse brain with tissue cysts was frozen for DNA extraction.

Genetic characterization for *Toxoplasma gondii*

A portion of lungs or brain was removed from mice that died within 4 wk after inoculation of chicken tissues and was frozen at −70 C for DNA characterization. *Toxoplasma gondii* DNA was extracted from mouse tissue as described previously (Lehmann et al., 2000). The RFLP strain type of *T. gondii* isolates was determined by nested polymerase chain reaction on the SAG2 locus according to Howe et al. (1997).

RESULTS

Democratic Republic of Congo

Antibodies to *T. gondii* were found in 25 of 50 chickens, with titers of 1:5 in 7, 1:10 in 7, 1:20 in 6, 1:40 in 1, and 1:160 or more in 4. *Toxoplasma gondii* was isolated from tissues of 10 chickens: from 1 with a titer of 1:10, 4 with titer of 1:20, 1 with titer of 1:40, and 4 with titer of 1:160. Nine *T. gondii* isolates were from chickens whose tissues were bioassayed individually: 3 isolates were from heart alone, 3 were from heart and brain, and 3 were from hearts and skeletal muscle (Table I). The isolates of *T. gondii* from chickens nos. 1 and 17 killed all infected mice 9–18 days PI. Genetically, 8 of these isolates were type III and 1 was type I (Table I).

The 10th isolate (isolate designation TgCkDROC-10) of *T. gondii* was from 1 of 5 mice inoculated with pooled tissues from chicken no. 10; this chicken had a MAT titer of 1:10. This isolate was avirulent for mice and was genotype II. *Toxoplasma gondii* was not found in the remaining 65 mice inoculated with tissues of the other 13 chickens that had a titer of 1:5 or 1:10. The cat fed tissues of 25 seronegative chickens did not shed oocysts.

Mali and Burkina Faso

Toxoplasma gondii was isolated from 5 of 48 chickens from Mali and 1 of 40 chickens from Burkina Faso (Table II). None of the infected mice died, indicating that these isolates were not virulent for mice. Genetically, 4 isolates from Mali were type III and 1 was type II, whereas the sole isolate from Burkina Faso was type II.

TABLE II. Isolation of *Toxoplasma gondii* from chickens from Mali, Burkina Faso, and Kenya.

Country	Chicken no.	No. of mice			Genotype (isolate designation)
		Inoculated	Dead	Positive for *T. gondii*	
Mali	1	5	0	1	II (TgCkMal-1)
Mali	10	5	0	1	III (TgCkMal-2)
Mali	15	5	0	1	III (TgCkMal-3)
Mali	32	5	0	2	III (TgCkMal-4)
Mali	34	5	0	2	II (TgCkMal-5)
Burkina Faso	36	3	0	3	III (TgCkBF-1)
Kenya	3	5	0	1	II (TgCkKen-1)

Kenya

Antibodies to *T. gondii* were found in 4 (1:100, 1:200, 1:200, and 1:800) of 30 chickens; the chicken with the titer of 1:800 had a prozone with no detectable antibody in 1:25 dilution of serum. Tissue cysts were found in the brain of 1 of 5 mice killed 56 days after inoculation with brain of the chicken with a titer of 1:800. All 5 mice subinoculated with brain homogenate of this Kenyan isolate of *T. gondii* had viable tissue cysts in their brains when killed 6 mo later; the genotype of this isolate was II.

DISCUSSION

The threshold MAT titer indicative of *T. gondii* infection in chickens has not been determined. Data comparing serology and recovery of viable *T. gondii* from chickens are now accumulating (Dubey et al., 2002; Dubey, Graham, Dahl, Hilali et al., 2003; Dubey, Graham, Dahl, Sreekumar et al., 2003; Dubey, Graham, da Silva et al., 2003; Dubey, Levy et al., 2004). In the present study, *T. gondii* was not isolated from chickens with titers of less than 1:10, and the likelihood of isolation increased with MAT titer. The MAT test used in this study is at present the best assay to detect antibodies to *T. gondii* in chickens (Dubey et al., 1993). Unlike in other hosts, the classic Sabin–Feldman dye test does not detect antibodies to *T. gondii* in chickens (Dubey et al., 1993). Ruiz and Frenkel (1980) isolated *T. gondii* from 27 of 54 chickens from Costa Rica that had no detectable dye test antibodies in 1:2 dilution of serum.

Notably, *T. gondii* was isolated from the hearts more frequently than from the brains or pectoral muscles of the chickens. In another recent study from our laboratories, *T. gondii* was isolated from hearts of 10 chickens and not from the brains of chickens from Peru (Dubey, Levy et al., 2004). *Toxoplasma gondii* is regarded as a neurotropic organism. However, this assumption is based on the studies in mice and because humans can develop severe neural toxoplasmosis. Studies in other asymptomatic animals have questioned this assumption (Jacobs et al., 1963; Dubey and Beattie, 1988; Dubey, 1997). Jacobs and Melton (1966) isolated *T. gondii* from 4 of 108 chickens from a slaughterhouse in Maryland: from ovaries of 3 and leg muscles of 1 but not from the brain of any chicken. Dubey (1981) isolated *T. gondii* from 3 of 11 free-range chickens from a farm in Montana; the parasite was detected in the hearts of all 3 and brain of 1 but not from their lungs, spleen, liver, kidneys, and pectoral muscles. In the present study, tissues from chickens from DROC were reasonably fresh and thus provided an opportunity to compare tissue tropism. Based on the results, it is clear from these studies that hearts should always be included among tissues when attempting to isolate *T. gondii* from chickens. The amount of material bioassayed did not affect the results because the entire brains and hearts were bioassayed; approximate weights of each brain, heart, and pectoral muscles bioassayed were 3, 5, and 10 g, respectively.

The mouse virulence and genotyping data indicate that the isolates of *T. gondii* from DROC are different from isolates from other countries. The lineage composition in DROC was apparently similar to that in Argentina (Dubey, Venturini et al., 2003) and apparently different from chickens from Brazil (Dubey et al., 2002; Dubey, Graham, da Silva et al., 2003; Dubey, Navarro et al., 2003; Lehmann et al., 2004). Most Brazilian isolates were virulent for mice, irrespective of the genotype, whereas 8 of 10 isolates from DROC were avirulent for mice. The distances among properties from where *T. gondii*–positive chickens were obtained indicates that isolates from DROC were independent. In contrast, none of the isolates from Egypt (Dubey, Graham, Dahl, Hilali et al., 2003), India (Sreekumar et al., 2003), Mexico (Dubey, Morales et al., 2004), or the United States (Dubey, Graham, Dahl, Sreekumar et al., 2003; Lehmann et al., 2003) was virulent for mice. Only a few isolates were available from Kenya, Mali, and Burkina Faso for genetic comparison. The low prevalence of viable *T. gondii* in chickens from Kenya was probably due to the low prevalence of *T. gondii* in the brains versus hearts of chickens (Dubey, Levy et al., 2004); only brains were available for parasite isolation. The low prevalence of *T. gondii* from chickens from Mali and Burkina Faso may, in part, be related to tissues autolysis before bioassay in mice.

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